Selective Feeding of Tobacco Budworm and Bollworm (Lepidoptera: Noctuidae) on Meridic Diet with Different Concentrations of *Bacillus thuringiensis* Proteins

J. GORE, J. J. ADAMCZYK, JR., AND C. A. BLANCO

Southern Insect Management Research Unit, USDA–ARS, P.O. Box 346, Stoneville, MS 38776

J. Econ. Entomol. 98(1): 88–94 (2005)

ABSTRACT Laboratory experiments were conducted to evaluate the behavior of bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), larvae on meridic diet with different concentrations of the Cry1Ac and Cry2Ab proteins from *Bacillus thuringiensis* subsp. *kurstaki* Berliner. The proteins used in these experiments are the ones in commercially available Bollgard and Bollgard II cotton. Both bollworms and tobacco budworms selectively fed on nontreated diet compared with diet treated with Cry1Ac. In addition, bollworms exhibited a concentration response with Cry1Ac. In general, bollworms selected diet with low concentrations of Cry1Ac compared with diet with higher concentrations of Cry1Ac. For Cry2Ab, the avoidance was not as prominent as that observed for Cry1Ac. Based on results from no-choice assays, the Cry1Ac and Cry2Ab concentrations used in choice assays represented a wide range of biological activity on both species. The lower concentrations provided low levels of mortality, whereas the higher concentrations provided high levels of mortality. Also, the developmental times of larvae were longer at higher concentrations of both proteins. These data provide important information about the behavioral response of key cotton pests to the *B. thuringiensis* proteins found in commercially available transgenic cotton. This information will be important to develop accurate scouting and management procedures for Bollgard and Bollgard II cotton.

KEY WORDS integrated pest management, insect behavior, Bollgard, Bollgard II

BIOTECHNOLOGY IS RAPIDLY CHANGING agriculture throughout the world. As new techniques are developed, the ability to insert new genes into crop plants expands. One of the most successful implementations of biotechnology has been the transformation of cotton plants to express insecticidal proteins from *Bacillus thuringiensis* subsp. *kurstaki* Berliner (Bt) (Perlak et al. 1991, 2001). Current commercial cultivars include Bollgard and Bollgard II (Monsanto Co., St. Louis, MO) that produce one or two insecticidal proteins, respectively, from *B. thuringiensis* (Adamczyk et al. 2001, Greenplate et al. 2003). Bollgard cotton was first sold for commercial production during the 1996 growing season and contains the *B. thuringiensis* gene that controls production of Cry1Ac (Greenplate 1999). Since it was first introduced, Bollgard cotton has continued to provide effective control of tobacco budworm, *Heliothis virescens* (F.), but insecticide applications have been needed for bollworm, *Helicoverpa zea* (Boddie), and other lepidopteran pests (Stewart et al. 2001). Applications for bollworms are typically needed during flowering stages of plant development when populations are moderate to high. Thus, Bollgard II cultivars were developed that produce two different proteins from *B. thuringiensis.* Bollgard II cultivars produce the Cry1Ac protein that is in the original Bollgard cultivars plus Cry2Ab (Greenplate et al. 2003). The addition of the second protein has significantly increased the level of control of bollworms and other lepidopteran pests (Adamczyk et al. 2001, Chitkowski et al. 2003, Gore et al. 2003, Jackson et al. 2003) and is an important aspect of resistance management (Greenplate et al. 2003).

Several factors contribute to the differences in the levels of control of bollworms and tobacco budworms in Bollgard cotton. Tobacco budworms are ≈20–30-fold more susceptible to Cry1Ac than bollworms (Luttrell et al. 1999). However, variability in expression of the protein among different plant parts and avoidance by the target insects also may be of some importance (Greenplate 1999, Adamczyk et al. 2001). For example, bollworm larvae are more mobile on Bollgard cotton than on conventional cotton (Gore et al. 2002). In general, bollworms avoid structures with high expression such as terminals and squares and selectively feed on tissues with lower expression such as white flowers and bolls (Greenplate 1999; Adamczyk et al. 2001; Gore et al. 2001, 2002). Similar results have been

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation by the USDA for its use.

observed with tobacco budworm on Bollgard plants (Benedict et al. 1992, 1993, 1994; Parker and Luttrell 1999). In addition, bollworms and tobacco budworms avoid *B. thuringiensis* proteins incorporated into meridic diet (Mohd-Salleh and Lewis 1982, Gould and Anderson 1991, Ashfaq et al. 2001). Tobacco budworms avoid sublethal concentrations of the *B. thuringiensis* Cry1Ac protein in meridic diet (Gould and Anderson 1991). However, little work has been done to determine whether bollworms avoid *B. thuringiensis* proteins at concentrations that produce low levels of mortality. Also, little work has been conducted to investigate bollworm or tobacco budworm behavior among different proteins. The objective of the current study was to determine whether there is a concentration effect on bollworm and tobacco budworm avoidance of the Cry1Ac and Cry2Ab proteins found in Bollgard and Bollgard II cotton.

Materials and Methods

Experiments were conducted in the laboratory to determine the feeding behavior of bollworms and tobacco budworms exposed to diet with a range of *B. thuringiensis* Cry1Ac and Cry2Ab protein concentrations that exhibited different levels of biological activity. The tobacco budworm colony used for these experiments was originally collected from velvetleaf, *Abutilon theophrasti* (L.), during May 2002. The bollworm colony was collected from field corn, *Zea mays* L., during June 2002. Approximately 2,000 larvae of each species were collected over a 2-wk period. Larvae were transported to the laboratory and fed a wheat germ-based meridic diet (King and Hartley 1985) in 29.5-ml plastic cups with matching lids. Larvae were maintained on meridic diet until pupation. Adults were maintained in 3.79-liter cardboard buckets (40–50 per bucket). The tops of the buckets were covered with batiste cloth to provide a surface for oviposition. Egg sheets were harvested daily and placed into 3.79-liter plastic bags. All stages were maintained in an environmentally controlled room at 27 ± 2°C, 80 ± 5% RH, and photoperiod of 14:10 (L:D) h. The colonies were in the laboratory for four to eight generations before testing.

Larval Choice Experiments. To determine feeding preference, each test arena consisted of a 50-mm-diameter self-sealing petri dish (BD Falcon No. 351006, VWR International, West Chester, PA). Four separate experiments were conducted. An experiment was defined by species and protein. Each experiment was arranged in a randomized complete block design with four replications. Replicates were temporally separated based on date of initiation (replicated over time). Each replicate consisted of 30 petri dishes. In total, six diet pellets were randomly arranged around the edge of each petri dish. The six diet pellets consisted of five different concentrations of the specific *B. thuringiensis* protein along with a nontreated pellet. The arrangement of concentrations around each arena remained consistent within a replicate, but it varied among replicates. The concentrations of each protein for each species are listed in Table 1. The concentrations chosen were based on preliminary bioassays (data not shown). Concentrations were selected to obtain a wide range of biological activity. For bioassays with Cry1Ac, lyophilized powder of MVPII (Monsanto Co.) containing 19.7% Cry1Ac by weight was incorporated into meridic diet (King and Hartley 1985) to obtain the desired concentrations. For Cry2Ab, lyophilized corn tissue (Monsanto Co.) containing 0.6% Cry2Ab by weight was used. The Cry1Ac in MVPII is an insoluble protoxin from a *Pseudomonas flourescens* formulation, whereas the Cry2Ab in freeze-dried corn tissue is expressed as a soluble toxin. Therefore, direct statistical comparisons between the two proteins are difficult.

Table 1. Concentrations of *B. thuringiensis* proteins used in bioassays

Concn in meridic diet (μg/ml)			
H. virescens		*H. zea*	
Cry1Ac	Cry2Ab	Cry1Ac	Cry2Ab
0	0	0	0
0.005	0.01	0.01	0.05
0.01	0.05	0.05	0.5
0.05	0.1	0.5	1.0
0.5	0.5	1.0	5.0
1.0	1.0	5.0	10.0

Meridic diet was poured into 100-mm-diameter petri dishes (BD Falcon No. 351029, VWR International) to a depth of 3 mm. A no. 3 cork borer was used to cut diet disks (8 mm in diameter by 3 mm in thickness) from each petri dish. One disk of each *B. thuringiensis* protein concentration was placed around the edge of each test arena according to the randomization scheme for each protein and species. A single neonate was placed in the center of each test arena and allowed to freely move around the arena. The locations of larvae were recorded in each experimental arena on each of five consecutive days. The percentages of larvae feeding on a specific concentration were calculated based on the total numbers of larvae per replication. Because the recordings were made on the same experimental units over time, the location of a larva in a specific dish is more closely correlated with the location on other dates within the same arena than between arenas. Therefore, a repeated measures analysis of variance (ANOVA) was used to analyze the percentage of larvae feeding on each concentration. Based on significance of the overall F-test, means were separated using the LSMEANS statement and adjusted according to the Tukey–Kramer test (PROC MIXED, SAS Institute, Cary, NC, Littell et al. 1996). For data where the rating interval by concentration interaction was significant, comparisons were only made for the main effects (rating period and concentration) because comparisons of different protein concentrations across rating periods are not important. For data where the main effects interactions were not significant, data for significant main effects were pooled across the other main effect. This occurred with Cry2Ab for both species, where the main effect

Table 2. Location of *H. virescens* larvae and reduction in weights of diet pellets within choice arenas for Cry1Ac (rating period*concn $F = 4.37$; df = 20, 72; $P < 0.01$)

Cry1Ac concn[a]	% ± SEM of larvae on diet at each rating period					% reduction in diet wt 120 h
	24 h	48 h	72 h	96 h	120 h	
0	26.0 ± 1.51a	22.5 ± 3.68a	19.1 ± 3.50a	34.0 ± 5.27a	33.7 ± 1.53a	43.3 ± 1.90a
0.005	5.9 ± 1.62bc	10.0 ± 3.87b	13.8 ± 3.70ab	21.0 ± 5.23b	16.8 ± 2.98b	41.5 ± 2.43ab
0.01	6.7 ± 1.35bc	7.5 ± 2.50b	10.3 ± 2.25b	14.5 ± 1.68b	10.3 ± 3.37bc	41.4 ± 1.17ab
0.05	2.5 ± 0.83c	10.9 ± 2.49b	7.8 ± 1.62b	3.0 ± 1.00c	5.3 ± 2.03c	37.5 ± 0.99b
0.5	3.4 ± 1.37c	9.2 ± 2.10b	6.2 ± 2.29b	4.0 ± 1.70c	3.2 ± 1.07c	32.4 ± 1.67c
1.0	11.0 ± 1.65b	8.3 ± 5.00b	12.0 ± 2.05ab	0.0 ± 0.00[b]	3.2 ± 1.05c	31.4 ± 1.09c

Means within a column followed by the same letter are not significantly different according to the Tukey-Kramer test ($\alpha = 0.05$).
[a] Micrograms of protein per milliliter of meridic diet.
[b] Values of zero were excluded from statistical analyses.

for protein concentration was significant, but the main effect for rating period was not.

In addition to larval location, the weights of each diet pellet were determined and recorded before and after each experiment. The percent reduction in weight was calculated for each diet pellet to provide an estimate of the amount of diet consumed. Weight reduction from natural water loss was not accounted for; however, the relative difference between the different concentrations should provide a good estimate of consumption. Percent reduction in diet weight was analyzed with ANOVA and means were separated using LSMEANS and adjusted according to the Tukey–Kramer test (PROC MIXED, SAS Institute, Cary, NC; Littell et al. 1996).

No-Choice Experiments. Bollworms and tobacco budworms were exposed to each concentration of *B. thuringiensis* protein used in the choice experiments (Table 1). Tobacco budworm and bollworm neonates were placed individually on ≈15 ml of meridic diet in 29.5-ml plastic cups. Thirty larvae were placed on each concentration on each of 4 d (replicates). The dates corresponded with those of the choice experiment so that results would closely reflect the response of each cohort of insects. Meridic diet was obtained from the same batch of diet used in the choice experiments to maintain homogeneity between the two experiments. Mortality was rated at 7 d. Surviving larvae were allowed to complete development. Larval development recorded as time to pupation and total mortality at the time of pupation was recorded. Time to pupation and mortality were analyzed with ANOVA, and means were separated with the LSMEANS statement and adjusted according to the Tukey–Kramer test (PROC MIXED, SAS Institute; Littell et al. 1996).

Results

Larval Choice Experiments. Tobacco budworm and bollworm larvae selected nontreated diet over diet treated with Cry1Ac (Tables 2 and 3). For tobacco budworm, there was a concentration by rating period interaction ($F = 4.37$; df = 20, 72; $P < 0.01$) for Cry1Ac (Table 2). Tobacco budworms generally selected nontreated diet over treated diet at all rating periods. Additionally, the percentage of larvae feeding on nontreated diet tended to increase at the later rating periods. At the 24-h rating period, 26.0% of larvae were on the nontreated diet, whereas the percentage of larvae on specific concentrations of treated diet ranged from 3.4 to 11.0. At the 120-h rating period, 33.7% of larvae were on the nontreated diet. Among the different concentrations of Cry1Ac-treated diet, tobacco budworms did not seem to show a high level of selection except at the 96- and 120-h rating periods.

Although the locations of larvae did not show a high level of selection among the treated diet, tobacco budworms did show some selection among the different concentrations of Cry1Ac ($F = 9.66$; df = 5, 18; $P < 0.01$) based on reductions in diet weights (Table 2). The weight reductions were highest for nontreated diet pellets and diet pellets with the two lowest concentrations of Cry1Ac (0.005 and 0.01 μg/ml). Also, the percentage of reduction in diet weights was lowest for the diet pellets with the two highest concentrations of Cry1Ac (0.5 and 1.0 μg/ml).

Table 3. Location of *H. zea* larvae and reduction in weights of diet pellets within choice arenas for Cry1Ac (rating period*concn $F = 1.80$; df = 20, 72; $P = 0.04$)

Cry1Ac concn[a]	% (± SEM) of larvae on diet at each rating period					% reduction in diet wt 120 h
	24 h	48 h	72 h	96 h	120 h	
0	28.3 ± 5.20a	25.5 ± 1.85a	26.4 ± 5.01a	37.6 ± 3.66a	37.9 ± 6.86a	40.7 ± 2.62a
0.01	16.7 ± 4.52b	22.2 ± 3.16ab	19.7 ± 4.62ab	14.1 ± 6.09b	11.6 ± 3.17bc	40.3 ± 0.88a
0.05	12.5 ± 3.71bc	13.5 ± 1.37bc	13.8 ± 3.01bc	14.3 ± 1.60b	16.2 ± 2.31b	35.9 ± 0.83b
0.5	11.7 ± 4.40bc	14.3 ± 2.09bc	11.8 ± 3.13bc	8.0 ± 3.38bc	8.1 ± 1.00bc	33.7 ± 1.05bc
1.0	5.0 ± 2.16c	5.0 ± 0.97cd	10.2 ± 1.32c	8.9 ± 1.79bc	7.2 ± 3.88bc	32.6 ± 1.22cd
5.0	4.2 ± 2.10c	3.3 ± 2.36d	6.8 ± 1.37c	2.7 ± 0.91c	4.4 ± 2.15c	30.6 ± 1.08d

Means within a column followed by the same letter are not significantly different according to the Tukey-Kramer test ($\alpha = 0.05$).
[a] Micrograms of protein per milliliter of meridic diet.

Table 4. Location of *H. virescens* larvae and reduction in weights of diet pellets within choice arenas for Cry2Ab (rating period*concn $F = 1.19$; df = 20, 72; $P = 0.29$; rating period $F = 0.04$; df = 4, 72; $P = 0.99$; concn $F = 4.42$; df = 5, 18; $P = 0.01$)

Cry2Ab Concn[a]	% larvae on diet[b] (mean ± SEM)[c]	Diet wt (% reduction ± SEM)
0	18.8 ± 2.43a	37.3 ± 2.81a
0.01	13.9 ± 1.80ab	36.5 ± 2.76a
0.05	16.6 ± 2.30ab	32.7 ± 2.37ab
0.1	15.2 ± 1.97ab	32.3 ± 1.31abc
0.5	8.9 ± 1.57b	27.5 ± 1.57bc
1.0	7.9 ± 1.29b	26.4 ± 1.33c

[a] Micrograms of protein per milliliter of meridic diet.
[b] Means within a column followed by the same letter are not significantly different according to the Tukey-Kramer test ($\alpha = 0.05$).
[c] Data were pooled across rating periods.

Table 5. Location of *H. zea* larvae and reduction in weights of diet pellets within choice arenas for Cry2Ab (rating period*concn $F = 1.31$; df = 20, 72; $P = 0.20$; rating period $F = 0.47$; df = 4, 72; $P = 0.76$; concn $F = 6.30$; df = 5, 18; $P < 0.01$)

Cry2Ab Concn[a]	% larvae on diet[b] (mean ± SEM)[c]	Diet wt (% reduction ± SEM)
0	14.9 ± 1.98b	37.7 ± 2.62a
0.05	18.1 ± 2.67ab	36.2 ± 1.59ab
0.5	13.2 ± 2.11b	32.6 ± 2.90ab
1	31.7 ± 2.85a	29.6 ± 2.56bc
5	10.2 ± 3.30b	29.8 ± 2.97bc
10	8.3 ± 1.34b	26.8 ± 2.10c

[a] Micrograms of protein per milliliter of meridic diet.
[b] Means within a column followed by the same letter are not significantly different according to the Tukey-Kramer test ($\alpha = 0.05$).
[c] Data were pooled across rating periods.

There was a concentration by rating period interaction ($F = 1.80$; df = 20, 72; $P = 0.04$) for bollworms on Cry1Ac (Table 3). Similar to tobacco budworm, bollworms generally selected nontreated diet over diet treated with Cry1Ac. The percentage of larvae feeding on nontreated diet tended to increase over time. At 24 h, 28.3% of larvae were on nontreated diet, whereas 37.9% of larvae were on nontreated diet at 120 h. Additionally, bollworms showed a concentration response at all rating periods. In general, bollworms selected diet with low concentrations of Cry1Ac over diets with high concentrations of Cry1Ac. The percentage of bollworm larvae on the Cry1Ac-treated diet pellets generally decreased with increasing concentrations at all rating periods.

Reductions in weights of diet pellets further supported selection of nontreated diet and diet with low concentrations of Cry1Ac over diets with high concentrations of Cry1Ac ($F = 17.00$; df = 5, 18; $P < 0.01$) (Table 3). The percentage of reduction in diet weights was greatest for the nontreated diet pellets and diet pellets treated with the lowest concentration of Cry1Ac (0.01 μg/ml). The diet pellets with the two highest concentrations of Cry1Ac (1.0 and 5.0 μg/ml) had the lowest reductions in weight.

For tobacco budworm, there was not a Cry2Ab concentration by rating period interaction ($F = 1.19$; df = 20, 72; $P = 0.29$). There was a significant main effect for Cry2Ab concentration ($F = 4.42$; df = 5, 18; $P = 0.01$) but not for rating period ($F = 0.04$; df = 4, 72; $P = 0.99$). Therefore, data for the percentages of larvae feeding on the different concentrations were pooled across rating periods (Table 4). In general, tobacco budworms did not show a high level of avoidance of Cry2Ab. When pooled across rating periods, the percentage of tobacco budworms feeding on nontreated diet was greater than the percentage feeding on diet with the two highest concentrations (0.5 and 1.0 μg/ml) of Cry2Ab.

Although little selection was detected based on larval location, reductions in weights of the diet pellets indicated that some selection for nontreated diet and diet treated with low concentrations of Cry2Ab did occur ($F = 4.45$; df = 5, 18; $P = 0.01$) (Table 4). The reductions ranged from 37.3% for the nontreated diet to 26.4% for the diet treated with the highest concentration (1.0 μg/ml) of Cry2Ab.

Similar to tobacco budworm, bollworms did not exhibit a Cry2Ab concentration by rating period interaction ($F = 1.31$; df = 20, 72; $P = 0.21$) (Table 5). There was a significant main effect for Cry2Ab concentration ($F = 6.30$; df = 5, 18; $P < 0.01$) but not for rating period ($F = 0.47$; df = 4, 72; $P = 0.76$). Therefore, data were pooled across rating periods. In general, bollworms did not seem to show a high level of avoidance of the Cry2Ab protein.

Although larval location did not indicate a high level of bollworm avoidance, reductions in weights of diet pellets did indicate some selection for nontreated diet and diet treated with low concentrations of Cry2Ab ($F = 2.83$; df = 5, 18; $P = 0.05$) (Table 5). The reductions in diet weights ranged from 37.7% for the nontreated diet to 26.8% for diet pellets treated with the highest concentration (10.0 μg/ml) of Cry2Ab.

No-Choice Experiments. The concentrations of Cry1Ac and Cry2Ab used in the choice experiments exhibited varying levels of biological activity on both tobacco budworm and bollworm in no-choice experiments (Tables 6–8). At 7 d, the different concentrations of Cry1Ac produced varying levels of mortality for both tobacco budworm ($F = 59.1$; df = 5, 15; $P < 0.01$) and bollworm ($F = 52.6$; df = 5, 15; $P < 0.01$) (Table 6). Similar results were observed with Cry2Ab on tobacco budworm ($F = 11.36$; df = 5, 15; $P < 0.01$) and bollworm ($F = 3.55$; df = 5, 15; $P = 0.03$). In general, the highest concentrations of each protein resulted in the highest levels of mortality. The lowest concentrations of each protein resulted in low levels of mortality that were similar to mortality on nontreated diet.

There was a significant effect on tobacco budworm development for Cry1Ac ($F = 5.87$; df = 4, 12; $P = 0.01$) and Cry2Ab ($F = 18.84$; df = 5, 11; $P < 0.01$), and on bollworm development for Cry1Ac ($F = 40.01$; df = 5, 17; $P < 0.01$) and Cry2Ab ($F = 5.45$; df = 5, 12; $P < 0.01$) (Table 7). Based on time from larval eclosion to pupation, tobacco budworm and bollworm larvae generally completed development faster on nontreated diet than on treated diet. On the nontreated diet, development of tobacco budworm and bollworm lar-

Table 6. Mortality of larvae after 7 d on meridic diet with different concentrations of *B. thuringiensis* proteins in a no-choice bioassay

Protein concn[a]	% mortality ± SEM			
	H. virescens		*H. zea*	
	Cry1Ac	Cry2Ab	Cry1Ac	Cry2Ab
0	1.3 ± 1.25d	0.0 ± 0.00[b]	1.3 ± 1.25d	1.7 ± 1.68b
0.005	1.3 ± 1.25d	NA[c]	NA	NA
0.01	14.0 ± 2.61d	3.3 ± 4.7b	2.3 ± 2.25d	NA
0.05	32.5 ± 12.3c	7.1 ± 4.46b	8.3 ± 3.84cd	5.8 ± 3.43b
0.1	NA	13.0 ± 8.26b	NA	NA
0.5	54.0 ± 3.19b	37.3 ± 8.39ab	21.0 ± 5.96c	9.2 ± 7.12ab
1.0	67.0 ± 4.49a	68.2 ± 16.83a	46.5 ± 3.93b	8.3 ± 7.27ab
5.0	NA	NA	70.0 ± 4.43a	10.0 ± 6.39ab
10.0	NA	NA	NA	25.8 ± 10.03a
$P > F$	<0.01	<0.01	<0.01	0.03

Means within a column followed by the same letter are not are not significantly different according to the Tukey-Kramer test ($\alpha = 0.05$).
[a] Micrograms of protein per milliliter of meridic diet.
[b] Data were excluded from analyses because no mortality was observed.
[c] Concentration of the protein was not used for this species.

vae ranged from 11.3 to 16.5 d. Development on the highest concentrations of treated diet ranged from 19.9 d for bollworms on diet treated with 10 μg/ml of Cry2Ab to 32.3 d for bollworms on diet treated with 5.0 μg/ml Cry1Ac. No tobacco budworms completed development on diet with Cry1Ac at 1.0 μg/ml, whereas the development time for tobacco budworms on 1.0 μg/ml Cry2Ab averaged 21.5 d.

Mortality at the time of pupation for tobacco budworms and bollworms varied in response to Cry1Ac and Cry2Ab concentrations in diet (Table 8). There was a significant effect on tobacco budworm mortality for Cry1Ac ($F = 57.05$; df = 5, 18; $P < 0.01$) and Cry2Ab ($F = 40.53$; df = 5, 18; $P < 0.01$), and on bollworm mortality for Cry1Ac ($F = 40.01$; df = 5, 17; $P < 0.01$) and Cry2Ab ($F = 5.45$; df = 5, 12; $P < 0.01$). Mortality on nontreated diet ranged from 0.8 to 4.6%. Mortality of both species was low to moderate (9.3–27.5%) on the lowest concentration of the two proteins. In general, mortality was very high (87.5–100.0%) on the highest concentrations with the exception of bollworms on the highest concentration of Cry2Ab (67.0%).

Discussion

Tobacco budworms and bollworms showed some level of avoidance of *B. thuringiensis* Cry1Ac and Cry2Ab proteins in these assays. This confirms results of previous studies that demonstrate tobacco budworm and bollworm avoidance of various *B. thuringiensis* proteins (Mohd-Salleh and Lewis 1982, Gould and Anderson 1991, Ashfaq et al. 2001). Based on larval location, neonates in the current study tended to show a stronger avoidance of Cry1Ac than Cry2Ab. Also, there was a concentration response for bollworm avoidance of Cry1Ac. This response was not apparent with neonate bollworms on Cry2Ab or with neonate tobacco budworms on Cry1Ac or Cry2Ab based on larval location. One possible reason for this is that bollworms regurgitate diet containing Cry1Ac faster than diet containing Cry2Ab (English et al. 1994). Based on reductions in diet weights, both species did seem to show selection among the different concentrations of the two proteins. However, data for percentage of reductions in diet weights should be interpreted cautiously because feeding rates of an

Table 7. Development of larvae on meridic diet with concentrations of the proteins used in the no-choice bioassays

Protein Concn[a]	Time to pupation ± SEM (d)			
	H. virescens		*H. zea*	
	Cry1Ac	Cry2Ab	Cry1Ac	Cry2Ab
0	16.5 ± 2.79b	11.3 ± 0.59d	16.1 ± 0.67d	11.9 ± 0.45c
0.005	17.1 ± 2.23b	NA[b]	NA	NA
0.01	19.4 ± 1.50ab	12.3 ± 0.46cd	20.1 ± 0.88c	NA
0.05	25.8 ± 0.58a	14.2 ± 0.79bc	22.5 ± 1.15c	13.5 ± 0.53bc
0.1	NA	15.3 ± 0.1.13b	NA	NA
0.5	32.0 ± 0.00[c]	18.8 ± 1.10a	26.7 ± 0.74b	16.0 ± 1.08ab
1.0	0.0 ± 0.00[d]	21.5 ± 0.25a	27.9 ± 1.08b	17.2 ± 1.67ab
5.0	NA	NA	32.3 ± 0.37a	18.2 ± 1.34a
10.0	NA	NA	NA	19.9 ± 1.87a
$P > F$	0.01	<0.01	<0.01	0.01

Means within a column followed by the same letter are not are not significantly different according to the Tukey-Kramer test ($\alpha = 0.05$).
[a] Micrograms of protein per milliliter of meridic diet.
[b] Concentration of the protein was not used for this species.
[c] Two larvae pupated at 32 d. Data were excluded from analyses because of a lack of variability.
[d] Data were excluded from analyses because no larvae reached pupation.

Table 8. Mortality of larvae at the time of pupation on meridic diet with different concentrations of *B. thuringiensis* proteins in a no-choice bioassay

Protein concn[a]	% mortality ± SEM[b]			
	H. virescens		*H. zea*	
	Cry1Ac	Cry2Ab	Cry1Ac	Cry2Ab
0	4.6 ± 1.79d	0.8 ± 0.75d	3.3 ± 1.44e	2.5 ± 1.66d
0.005	19.0 ± 5.45cd	NA[b]	NA	NA
0.01	23.9 ± 9.90c	8.8 ± 5.54cd	27.5 ± 9.6d	NA[c]
0.05	43.1 ± 6.81b	9.3 ± 3.50cd	41.5 ± 4.3cd	9.3 ± 3.71cd
0.1	NA	19.3 ± 7.45c	NA	NA
0.5	98.3 ± 1.68a	57.0 ± 7.52b	59.0 ± 2.45b	20.3 ± 5.82bc
1.0	100.0 ± 0.00[c]	87.5 ± 4.33a	57.5 ± 6.41bc	27.8 ± 6.37b
5.0	NA	NA	91.0 ± 4.60a	30.5 ± 8.02b
10.0	NA	NA	NA	64.0 ± 8.34a
$P > F$	<0.01	<0.01	<0.01	<0.01

Means within a column followed by the same letter are not are not significantly different according to the Tukey-Kramer test ($\alpha = 0.05$).
[a] Micrograms of protein per milliliter of meridic diet.
[b] Concentration of the protein was not used for this species.
[c] Data were excluded from analyses because no larvae survived to pupation.

individual larva would be higher on lower concentrations than on higher concentrations. Although larval location was only recorded at discrete times, it probably provides a better estimate of larval response to the *B. thuringiensis* proteins. The concentration response of bollworms to Cry1Ac could have a significant impact on the pest status of bollworms on Bollgard cotton, because levels of Cry1Ac vary among different plant parts (Greenplate 1999, Adamczyk et al. 2001). This coupled with increased mobility of bollworms in Bollgard cotton (Gore et al. 2002) indicates that if larvae are able to select diet with Cry1Ac concentrations at sublethal levels, they will have an increased chance for survival in Bollgard cotton. In Bollgard cotton, bollworms preferentially feed in white flowers (Gore et al. 2002) where their survival is greatest. Therefore, this behavior may partially explain why some Bollgard fields have to be treated for bollworms but not for tobacco budworms. Although tobacco budworms avoid the Cry1Ac protein, they do not seem capable of distinguishing between different concentrations as neonates as first suggested by Gould and Anderson (1991). Because all Bollgard plant structures contain some level of Cry1Ac, tobacco budworms are not likely to settle on a particular structure for feeding, whereas bollworms may be able to distinguish and selectively feed on structures with low expression. Despite the differences in behavior, the most apparent reason that Bollgard fields need to be treated for bollworms and not tobacco budworms is because bollworms are much less susceptible to Cry1Ac than tobacco budworms (Luttrell et al. 1999).

Direct comparisons between species and proteins are difficult because they were evaluated in different experiments and different forms of the proteins were used (i.e., insoluble Cry1Ac versus soluble Cry2Ab). Also, the different concentrations used in the choice assays had different levels of biological activity in the no-choice assays. For instance, both species developed slower on high concentrations than on low concentrations of the proteins. Developmental rates on the nontreated diet were longer for both species in the Cry2Ab experiment than in the Cry1Ac experiment. Reasons for this are unknown; however, the Cry2Ab experiments were conducted after the Cry1Ac experiments and may be the result of the colonies being in the laboratory for more generations.

The difference in biological activity of the two proteins is apparent with mortality as well. Mortality of bollworms on the highest concentration of Cry2Ab averaged 64.0%, whereas mortality of bollworms on the highest concentration of Cry1Ac averaged 91.0%. Also, the lowest concentrations of Cry2Ab tended to produce lower levels of mortality than the lowest concentrations of Cry1Ac for both species. However, a behavioral response tended to be more apparent to different concentrations of Cry1Ac than to different concentrations of Cry2Ab. Cry2A proteins have less impact on tobacco budworms and bollworms than Cry1A proteins (English et al. 1994, Sims 1997, Perlak et al. 2001). Thus, differences in the behavioral response would be expected between the two proteins (English et al. 1994). Also, the Cry2Ab protein used in these experiments was derived from freeze-dried corn tissue, a preferred food source for bollworm (Johnson et al. 1975). Therefore, any avoidance of the Cry2Ab protein could have been masked by the presence of secondary plant chemicals in the corn tissue that may act as feeding stimulants for bollworms. In a separate experiment (data not shown), bollworms did not show a preference for meridic diet with freeze-dried, non-Bt corn tissue compared with meridic diet without the corn tissue ($F = 3.23$; df = 2, 6; $P = 0.11$).

These experiments evaluate the behavior of target insects to the *B. thuringiensis* Cry1Ac and Cry2Ab proteins in commercial transgenic varieties. Although no direct statistical comparisons can be made, the proteins seem to produce different behavioral responses, especially with bollworms. However, additional research will be needed to compare behavior among larvae fed different proteins at concentrations with similar biological activity. Differences in behavioral response could potentially impact the level of efficacy of cotton cultivars that have been genetically engineered to produce these

proteins. Currently, Bollgard and Bollgard II are the only commercially available insect resistant transgenic cultivars of cotton. However, new cultivars such as Widestrike (Dow AgroSciences, Indianapolis, IN) and VIPCOT (Syngenta Crop Protection, Wilmington, DE) are expected to be available in the near future. A thorough understanding of the behavioral mechanisms leading to the responses of bollworms and tobacco budworms to the proteins in these new varieties is needed to develop appropriate management strategies.

Acknowledgments

We thank Michelle Mullen, Les Price, Don Hubbard, and Jennifer Holcomb for assistance with these experiments. Critical reviews of this manuscript provided by Livy Williams, Jarrod Leland, and John Greenplate are appreciated.

References Cited

Adamczyk, J. J., Jr., L. C. Adams, and D. D. Hardee. 2001. Field efficacy and seasonal expression profiles for terminal leaves of single and double *Bacillus thuringiensis* toxin cotton genotypes. J. Econ. Entomol. 94: 1589–1593.

Ashfaq, M., S. Y. Young, and R. W. McNew. 2001. Bollworm (Lepidoptera: Noctuidae) development and movement on *Bacillus thuringiensis*-treated cotton leaves. J. Entomol. Sci. 36: 23–33.

Benedict, J. H., D. W. Altman, P. F. Umbeck, and D. R. Ring. 1992. Behavior, growth, survival, and plant injury by *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) on transgenic Bt cottons. J. Econ. Entomol. 85: 589–593.

Benedict, J. H., E. S. Sachs, D. W. Altman, D. R. Ring, T. B. Stone, and S. R. Sims. 1993. Impact of δ-endotoxin-producing transgenic cotton on insect-plant interactions with *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). Environ. Entomol. 22: 1–9.

Benedict, J. H., E. S. Sachs, J. L. Halcomb, D. R. Ring, B. Cook, D. M. Stelly, D. W. Altman, R. J. Kohel, J. C. Correa, M. W. Goynes, et al. 1994. Feeding preference and movement of tobacco budworm and bollworm in mixed stands of transgenic Bt and non-Bt cotton. Annu. Plant Resistance Insects Newsl. 20: 31–32.

Chitkowski, R. L., S. G. Turnipseed, M. J. Sullivan, and W. C. Bridges, Jr. 2003. Field and laboratory evaluations of transgenic cottons expressing one or two *Bacillus thuringiensis* var. *kurstaki* Berliner proteins for management of noctuid (Lepidoptera) pests. J. Econ. Entomol. 96: 755–762.

English, L., H. L. Robbins, M. A. von Tersch, C. A. Kulesza, D. Ave, D. Coyle, C. S. Jany, and S. L. Slatin. 1994. Mode of action of CryIIA: a *Bacillus thuringiensis* delta-endotoxin. Insect Biochem. Mol. Biol. 24: 1025–1035.

Gore, J., B. R. Leonard, and J. J. Adamczyk. 2001. Bollworm (Lepidoptera: Noctuidae) survival on Bollgard and Bollgard II cotton flower bud and flower components. J. Econ. Entomol. 94: 1445–1451.

Gore, J., B. R. Leonard, G. E. Church, and D. R. Cook. 2002. Behavior of bollworm (Lepidoptera: Noctuidae) larvae on genetically engineered cotton. J. Econ. Entomol. 95: 763–769.

Gore, J., Leonard, B. R., and R. H. Gable. 2003. Distribution of bollworm, *Helicoverpa zea* (Boddie), injured reproductive structures on genetically engineered *Bacillus thuringiensis* var. *kurstaki* Berliner cotton. J. Econ. Entomol. 96: 699–705.

Gould, F., and A. Anderson. 1991. Effects of *Bacillus thuringiensis* and HD-73 delta-endotoxin on growth, behavior, and fitness of susceptible and toxin adapted strains of *Heliothis virescens* (Lepidoptera: Noctuidae). Environ. Entomol. 20: 30–38.

Greenplate, J. T. 1999. Quantification of *Bacillus thuringiensis* insect control protein Cry1Ac over time in Bollgard cotton fruit and terminals. J. Econ. Entomol. 92: 1377–1383.

Greenplate, J. T., J. W. Mullins, S. R. Penn, A. Dahm, B. J. Reich, J. A. Osborn, P. R. Rahn, L. Ruschke, and Z. W. Shappley. 2003. Partial characterization of cotton plants expressing two toxin proteins from *Bacillus thuringiensis:* relative toxin contribution, toxin interaction, and resistance management. J. Appl. Entomol. 127: 340–347.

Jackson, R. E., J. R. Bradley, Jr., and J. W. Van Duyn. 2003. Field performance of transgenic cottons expressing one or two *Bacillus thuringiensis* endotoxins against bollworm, *Helicoverpa zea* (Boddie). J. Cotton Sci. 7: 57–64. http://journal.cotton.org.

Johnson, M. W., R. E. Stinner, and R. L. Rabb. 1975. Ovipositional response of *Heliothis zea* (Boddie) to its major hosts in North Carolina. Environ. Entomol. 4: 291–297.

King, E. G., and G. G. Hartley. 1985. *Diatraea saccharalis,* pp. 265–270. *In* P. Singh and R. F. Moore [eds.], Handbook of insect rearing, vol. 2. Elsevier, Amsterdam, The Netherlands.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS system for mixed models. SAS Institute, Cary, NC.

Luttrell, R. G., L. Wan, and K. Knighten. 1999. Variation in susceptibility of noctuid (Lepidoptera) larvae attacking cotton and soybean to purified endotoxin proteins and commercial formulations of *Bacillus thuringiensis.* J. Econ. Entomol. 92: 21–32.

Mohd-Salleh, M. B., and L. C. Lewis. 1982. Feeding deterrent response of corn insects to β-exotoxin of *Bacillus thuringiensis.* J. Invertebr. Pathol. 39: 323–328.

Parker, C. D., Jr., and R. G. Luttrell. 1999. Interplant movement of *Heliothis virescens* (Lepidoptera: Noctuidae) larvae in pure and mixed plantings of cotton with and without expression of the Cry1Ac δ-endotoxin protein of *Bacillus thuringiensis* Berliner. J. Econ. Entomol. 92: 837–845.

Perlak, F. J., R. L. Fuchs, D. A. Dean, S. L. McPherson, and D. A. Fischoff. 1991. Modification of the coding sequence enhances plant expression of insect control protein genes. Proc. Natl. Acad. Sci. U.S.A. 88: 3324–3328.

Perlak, F. J., M. Oppenhuizen, K. Gustafson, R. Voth, S. Sivasupramaniam, D. Heering, B. Carey, R. A. Ihrig, and J. K. Roberts. 2001. Development and commercial use of Bollgard cotton in the USA: early promises versus today's reality. Plant J. 27: 489–501.

Sims, S. R. 1997. Host activity spectrum of the CryIIA *Bacillus thuringiensis* subsp. *kurstaki* protein: effects on Lepidoptera, Diptera, and non-target arthropods. Southwest. Entomol. 22: 395–404.

Stewart, S. D., J. J. Adamczyk, Jr., K. S. Knighten, and F. M. Davis. 2001. Impact of Bt cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis* Berliner on growth and survival of noctuid (Lepidoptera) larvae. J. Econ. Entomol. 94: 752–760.

Received 23 June 2004; accepted 12 October 2004.